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Original Research Article

Restorative Potentials of Aqueous *Telfairia occidentalis* Seeds Extract on the Hippocampal Nissl Granules and Short-Term Memory in Scopolamine Hydrobromide-Induced Alzheimer's Type Cognitive Dysfunction Rats

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ABSTRACT

Cognitive dysfunction is one of the major health problems including Alzheimer's that is debilitating in nature. Neuroprotections are strategies and relative mechanisms that are able to defend the central nervous system against neuronal injury due to acute or chronic neurodegenerative disorders. This study elucidated the potentials of aqueous *Telfairia occidentalis* seed extract on Nissl substance in the hippocampus and short-term memory using scopolamine-induced Alzheimer's type cognitive dysfunction rats. With ethical approval from the Faculty of Basic Medical Sciences, University of Calabar, Calabar, Nigeria (FAREC-FBMS 042ANA3719), thirty Wistar rats weighing between 180-200 g were used for the study and grouped into five; A, B, C, D and E. Alzheimer's type cognitive dysfunction was induced in groups B through E before the extract and drug administration, followed by the novel object recognition test and histochemical tissue processing for Nissl granules demonstration with Cresyl Fast Violet stain. Neurobehavioral results revealed enhancement of short-term memory in the treated groups. The Nissl body stained with Cresyl Fast Violet under light microscope revealed less stain in group B. Groups C and E were mildly stained while Group D was deeply stained. The deeply stained Nissl granules in group D indicates increased protein synthesis which may cause proliferation of synapses in the hippocampal pyramidal cells hence, leading to enhanced learning and memory. In conclusion, aqueous extract of *Telfairia occidentalis* restored depleted hippocampal Nissl granules and enhanced short-term memory in scopolamine hydrobromide-induced Alzheimer's type cognitive dysfunction rats.

Keywords: Cognitive dysfunction, Hippocampus, Nissl bodies, Short term memory, *Telfairia occidentalis*, Wistar rats.

Introduction

Herbs are used therapeutically to provide support for different physiological systems including treatment and prevention of diseases in humans and animals.[1] These herbs may possess specific medicinal components such as polyphenols and carotenoids which are believed to minimize the risk of many major illnesses including cardiovascular diseases, cancer and neurodegenerative disorders. Hence, people who consume more vegetables and fruits may be at lower risk of developing diseases caused by neuronal dysfunction.[2,3] Extract from vegetables have been found to possess physiological use such as antioxidants angiotensin 1-converting enzyme inhibitors, antibiotics and cancer cell inhibitors.[4-10] Nutrients contained in vegetables seem to improve vascular health and mediate oxidative stress and inflammation, and consequently; have the ability to advance better cognitive health.[11] More so, dietary supplementations with fruits and vegetables have been shown to enhance cognitive disorder through biological processes.[12-14] A study has it that most of these plants act through receptors while some of them alter the availability of neurotransmitters in the brain. Therefore, cognitive enhancers are used primarily to treat people suffering from cognitive disorders such as Alzheimer's, Parkinson's and attention-deficit hyperactivity disorder.[15] Numerous human diseases are known to be related to free radicals. These free radical scavenging medicines are antioxidants in nature. The exogenous and endogenous antioxidants help to neutralize excess free radicals, protect cell against toxic effect and contribute to disease prevention.[16] The exogenous antioxidants are mainly derived from food and medicinal plants.[17] Natural antioxidants derived from medicinal plants are mainly polyphenols, carotenoids, vitamin C and lycopenes,[18] some of which are present in *Telfairia occidentalis* seeds. The nutritional value of *Telfairia occidentalis* seed is based on its high protein contents and high percentage of oil.[19] The oil includes fatty acids Oleic, linolenic, palmitic and stearic acid. It also contains 1% phytosterols, present in free and bound forms; squalene; chlorophyll pigments; minerals such as selenium, zinc, calcium, copper, iron, manganese, phosphorus and potassium; 30% pectins tocopherols; carotenoids, and also contain pharmacological properties like antidiabetic, antifungal, antibacterial, anti-inflammatory and antioxidant effects.[20,21] However, investigating the potentials of aqueous *Telfairia occidentalis* seed extract on the Nissl substance in the hippocampus as well as learning and memory using scopolamine hydrobromide-induced Alzheimer's type cognitive dysfunction rats,

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may yield deeper insight into their functionality, hence, the need for the present study.

Materials and Methods

Breeding of animals

Thirty adult female and male Wistar rats weighing between 180-200 g were bought from the University of Calabar. The animals were kept in the animal room in the Department of Anatomical Sciences, for two weeks under standard conditions of temperature (27^oC – 30^oC) for acclimatization. The animals were fed with rat chow (Agro Feed Mill Nigeria Limited, Calabar) and allowed access to drinking water *ad libitum*. After acclimatization, the experimental rats were randomly grouped into five, each containing six rats designated A, B, C, D and E. Ethical approval number: FAREC-FBMS 042ANA3719.

Plant extract preparation

Fresh *Telfairia occidentalis* seeds were obtained from Watt market, Calabar, Cross River State, Nigeria. The *Telfairia occidentalis* seeds were identified, authenticated and registered by a Botanist Mr Effa A. Effa with voucher number: HERB/BOT/UCC/322 in the Department of Botany, University of Calabar, Calabar. The plant seeds were removed from the shell, washed to free debris, chopped into smaller pieces and air-dried in the laboratory. The dried samples were blended into powder (model number: Bravo3JARS Mixer grinder) weighing 1600g and then soaked in 1000 mL of distilled water for twenty four-hours. The mixture was then filtered using chess cloth and Whatman No.1 filter paper. A solution was obtained and concentrated to a syrupy residue at 40^oC-50^oC using man-made vacuum (model number F.NR: 1508.0271) and kept in a cool dry place (0-8^oC) for later use.

Induction of Alzheimer's type cognitive dysfunction

Alzheimer's type cognitive dysfunction was induced in the adult female and male rats in groups B, C, D and E through daily intraperitoneal injection of 1.0 mg/kg body weight of scopolamine hydrobromide (SHB) for seven days.

Determination of LD_{50}

The LD50 of the *Telfairia occidentalis* seeds aqueous extract was established using the modified Lorke's method.22 Twenty-four adult Wistar rats weighing between 180-200 g were used for the first and second phases of the study. These rats were divided into 8 groups of 3 rats each. Three groups were used for each phase. The rats were fasted overnight before administering orally 500, 1000, 2000 and 4000 mg/kg body weight of *Telfairia occidentalis* seeds aqueous extract to the groups respectively, for the first phase with no signs of toxicity and mortality for seventy-two hours post administration. The second phase of the study was carried out using the remaining groups of animals; and was administered 4500, 5000, 6000 and 7000 mg/kg of aqueous *Telfairia occidentalis* seeds extract orally. Thereafter, the rats were monitored for signs of toxicity including mortality for 24, 48, 72 hours, one week and two weeks post administration. Hence, no signs of toxicity were observed, LD50 was established and the dosage of aqueous extract administration was determined using 12.5% and 25% of the established LD_{50}.

Plant extract and donepezil administration

Group A served as the positive control, received animal feed and water ad libitum; group B served as the negative control and received 1.0 mg/kg body weight of scopolamine hydrobromide (intraperitoneally) only; group C received 1.0 mg/kg body weight of SHB (intraperitoneally) and 1.0 mg/kg body weight of Donepezil (orally), group D received 1.0 mg/kg body weight of SHB (intraperitoneally) and 875 mg/kg body weight of aqueous *Telfairia occidentalis* seeds extract (orally) while group E received 1.0 mg/kg body weight of SHB (intraperitoneally) and 1750 mg/kg body weight of aqueous *Telfairia occidentalis* seeds extract(orally). The extract and Donpezil were administered for fourteen days.

Novel Object Recognition Task (NORT) procedure for short term memory

The Novel object recognition task in the rat is a facile assay for cognitive function. It is a relatively high-through-put, robust and sensitive procedure for evaluating compounds for cognitive enhancing activity. The novel object recognition test utilizes the open field maze. This consists of a square area surrounded by high walls (50 x 50 cm). The walls and floor were painted white, where the later was covered with plexi-glass. With the apparatus placed on the ground, the floor of the field was masked with gridlines (blue lines). These gridlines divided the area into equal smaller squares, helpful for scoring. A square area in the very centre of the area was also outlined with red line (centre square). The laboratory area was sufficient but dimly lit to allow animals to see and explore their surroundings while avoiding stress from bright lights. Two identical objects of same colour, texture and sizes were acquired and a novel object different from the identical objects also acquired.

Rats were brought to the neurobehavioral laboratory from the animal house in their home cages. Using the open field arena, a rat was picked and gently dropped on the centre square. The rats were given the opportunity to explore the two identical objects (one placed at Southwest and the other at Northeast during the exploration periods) for 5 minutes each; and later returned to the holding cages. During the habituation period, the rats were removed from its holding cages and placed in the middle of the empty open arena, where there were allowed to explore for 5 minutes, there after moved to the holding cages. After 5 minutes, the animals were then presented with two objects to explore, one of which was the same as in the first exploration trial, the other a new object for another 5 minutes. At the end of the 10 minutes, the rats were scored, picked and returned to their holding cages. Methylated spirit was used to thoroughly clean the open field arena to minimize olfactory cues. After 5 minutes interval, a new rat was introduced into the field.

Tissue processing and staining procedure

Twenty-four hours after the last administration, the animals were sacrificed; their brain tissues perfused and processed histochemically. Nissl granules in the hippocampal cells were stained using Cresyl Fast violet solution. The paraffin wax was removed from the sections in two changes of xylene for the period of 10 minutes. Each section was dehydrated in ascending grades of alcohol (70%, 95% and 100% or absolute alcohol) in two changes for 10 minutes each and rinsed in distilled water. Warm 0.1% Cresyl violet solution was used in staining the sections at a temperature of 37^oC. The sections were rinsed immediately in distilled water, differentiated in 95% alcohol for 30 minutes and then dehydrated in 100% alcohol for 10 minutes. There were cleared in xylene for 10 minutes and mount in Dibutylphthalate Polystyrene Xylene (DPX). The stained sections were observed under the light microscope.

Statistical analysis

Data obtained were statistically analyzed using analysis of variance (ANOVA) with the statistical package for social science (SPSS) version 22. Multiple comparisons were done using a two-way post hoc test with results expressed as mean ± standard error of mean (SEM) at $p < 0.05$.

Results and Discussion

Novel object recognition results

The frequency of exploration for objects A and B during habituation periods (trial 1, day 1) showed that the frequency of exploratory test of object A, group B was significantly lower when analogized with the control group A. Group E was significantly increased when compared with groups A, B, and C ($p < 0.05$). While the frequency of exploratory test of object B, group B showed significant decrease analogous to the control group A and also the group E was higher analogous to group A, B and C at $p < 0.05$ (Figure 1). During habituation period (trial 1, day 1), the duration of exploratory test in

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object A showed that groups C, D and E were significantly higher analogous to group B. The duration of exploratory test in object B shows that all the groups were not significantly different at $p < 0.05$ (Figure 2).

The frequency of exploratory test for objects A and B (trial 2, day 1) showed that frequency of exploratory test for object A, group D was significantly higher than other groups ($p < 0.05$). Similarly, group D showed meaningful elevation analogous to A, B, C and E. The frequency of exploratory test for object B showed that group D was elevated analogous to group A, B, C, and E whereas groups C and E were elevated analogous to group B at $p < 0.05$ (Figure 3). The result of duration of NORT for object A showed that group B decreased significantly analogous to the control group A while group D and E revealed significant increase analogous to the control group A and B. Groups A, C, D, and E increased significantly compared with group B. The duration in group C was longer compared to group B and shorter analogous to group E (Figure 4). The duration of NORT in object B showed that groups A, C, D and E were significantly higher when analogized with array B ($p < 0.05$). Array D and E revealed significant increase compared with arrays A, B and C ($p < 0.05$). Similarly, group C revealed a significant increase analogous with group B at $p < 0.05$ (Figure 4).

Histochemical result for Nissl granules

Cresyl fast violet stains of the hippocampal Nissl substance showed mild to moderate staining intensity in the treated experimental groups. The Nissl body demonstration using Cresyl fast violet stain revealed that group B treated with scopolamine alone was less stained in the pyramidal cells of the hippocampal rats (plate 2). Groups C, D, and E revealed deeply staining Nissl granules (plate 3, 4 and 5) compared to the group B treated with scopolamine alone and mild staining Nissl granules compared to the control group A (plate 1). The novel object recognition task (NORT) has become a widely used model for the investigation of memory alteration, configured to measure working memory, anxiety and preference for novelty in rodents.[23,24] Also, NORT has been used to test the effects of various pharmacological treatments and brain damage.[23] A mouse is presented with two similar objects during the first session and then one of the two objects is replaced by a new object during the second session. The amount of time spent to explore the new object provides an index of recognition memory.[25] The result from NORT is in line with similar works where *T. occidentalis* ameliorated karyorrhectic, atrophied and disrupted pyramidal cell membranes in the hippocampus as well as enhancing learning and memory in scopolamine hydrobromide-induced cognitive dysfunction rats using Morris water maze test.[26,27] Animals in the positive control administered with SHB alone, showed reduced frequency and duration in exploration of novel recognition compared to the negative control (Figures 3 and 4), indicating memory impairment. This result is similar to a study where SHB induced memory deficits and memory loss[28] which is usually the earliest sign of Alzheimer's disease. In the present study, animals in group D and E showed frequency increase and duration of exploration of novel object (figure 4). This may indicates that *T. occidentalis* has memory-enhancing activity against scopolamine-induced memory impairment. However, our result from the NORT established that SHB induced learning and memory impairment (figure 3) which may be due to oxidative stress induced by SHB.

Nissl bodies are granular materials found in neurons, mostly located in the cell bodies and dendrites and are responsible for synthesizing proteins. Disappearance of the Nissl granules may be due to exogenous insult or as a result of trauma.[29] In this study, it was observed that there was marked reduction of Nissl-stained neurons in the hippocampus of the rats treated with 1 mg/kg body weight of scopolamine hydrobromide alone (Plate 2), indicating reduction in protein synthesis. This study is in line with a similar report where chemicals and toxic substances affect Nissl substance thereby influencing their metabolic activities.[30] In the *T. occidentalis* groups, the animals that received 875 mg/kg body weight (Plate 5) and the animals treated with 1750 mg/kg body weight showed mild staining granules (Plate 4) compared with the normal control group A (Plate 1). Whereas, the group treated with 1 mg/kg body weight of donepezil also showed mild staining Nissl granules (Plate 3). The above results revealed that the aqueous extract of *T. occidentalis* administered at different dose ratios were able to offer protection against cellular damage of scopolamine hydrobromide. Many alterations that occur during loss of neuronal viability have been used as markers for dead cells. They include morphological and histochemical changes such as the development of basophilia, eosinophilia and loss of Nissl stain, shrinkage of the cell perikarya with the formation of angular-shaped neurons containing microvacuolations, dendritic swellings and accumulation of calcium deposits.

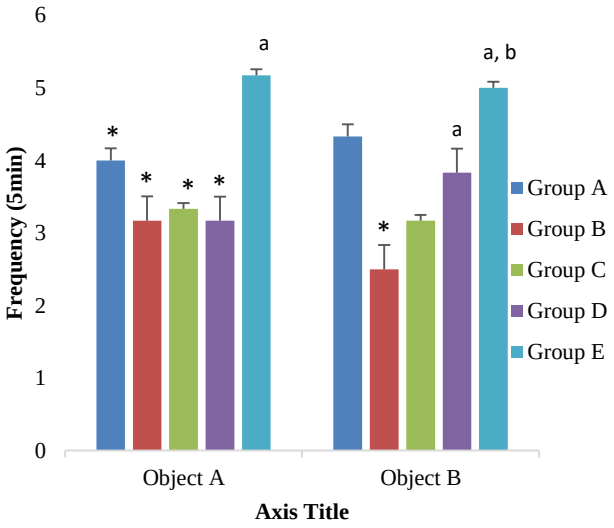

Figure 1: Comparison of frequency of line crossing in the different experimental groups during the Novel Object Recognition Task.

Values were set as mean ± SEM, n = 6, * = significant different control at $p < 0.05$. A = significant different group B at $p < 0.05$, b = significant different group C at $p < 0.05$.

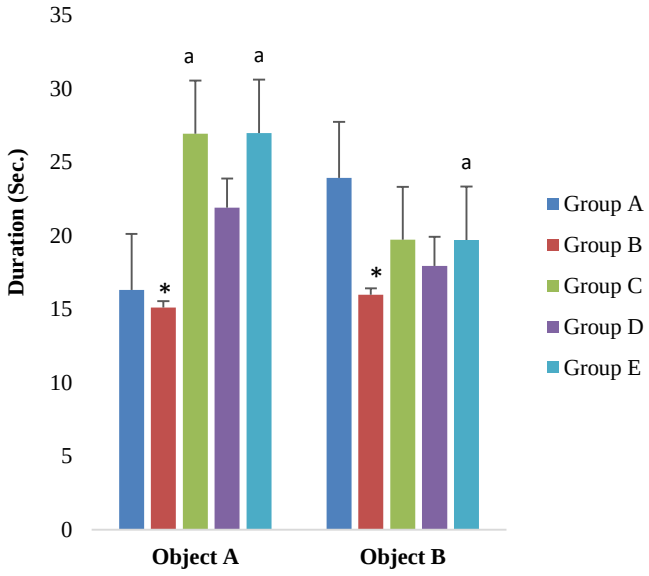

Figure 2: frequency of exploration test during habituation period (trial 1, day 1).

Values were seen as mean ± SEM, n = 6, * =significant different control at $p < 0.05$. A = significant different group B at $p < 0.05$.

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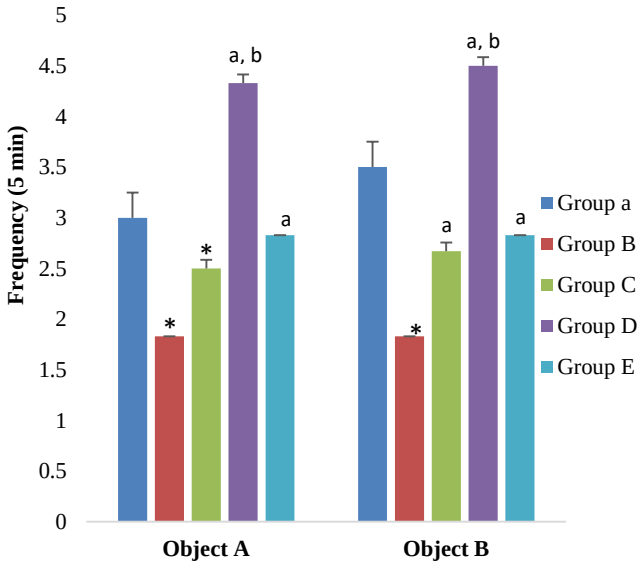

Figure 3: Frequency of exploration test during the test period (trial 2, day 1).
Data are shown as mean ± SEM, n = 6, * = significant different control at $p < 0.05$. A = significant different group B at $p < 0.05$, B = significant different group C at $p < 0.05$.

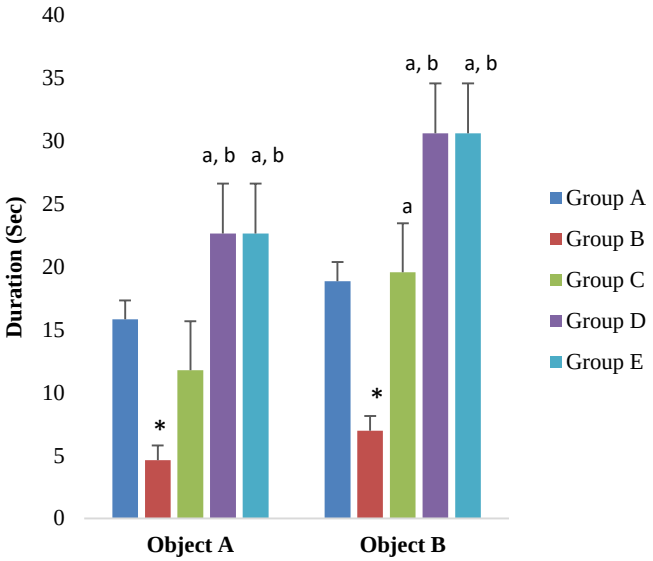

Figure 4: duration of exploration in NORT during the test period (trial 2, day 1).
Values were seen as mean ± SEM, n = 6, * = significant different control at $p < 0.05$. A = significant different group B at $p < 0.05$, B = significant different group C at $p < 0.05$.

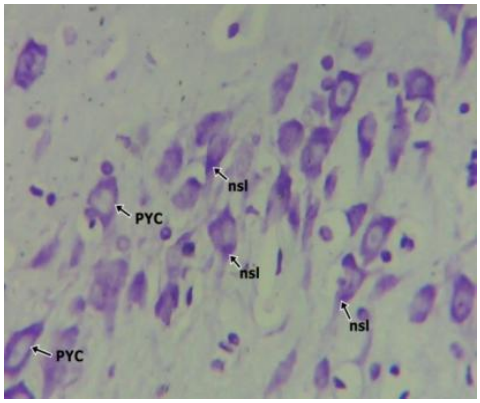

Plate 1: Photomicrograph of a section of hippocampus from group A shows deeply stained Nissl (NSL) substance in the pyramidal cells (PYC) with granular

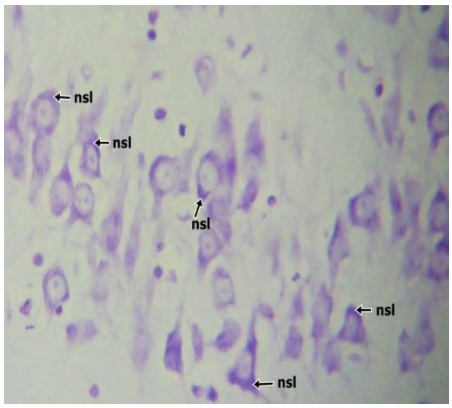

Plate 2: Photomicrograph of a section of hippocampus from group B shows less stained Nissl substance (NSL) in the pyramidal cell.

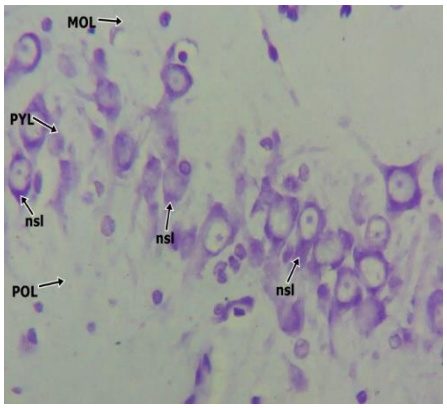

Plate 3: Photomicrograph of a section of hippocampus from group C shows abundant Nissl substance within the cytoplasm and more at the periphery of the pyramidal cell body.

V

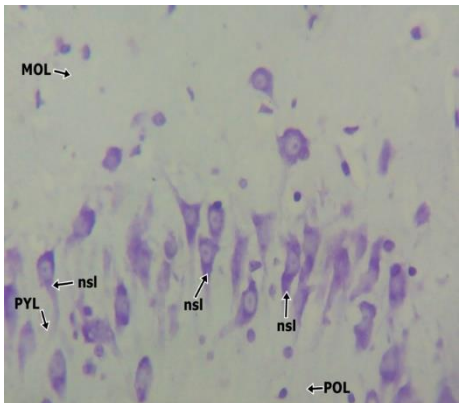

Plate 4: Photomicrograph of a section of hippocampus from group D shows even distribution and densely stained Nissl granules within their cytoplasm.

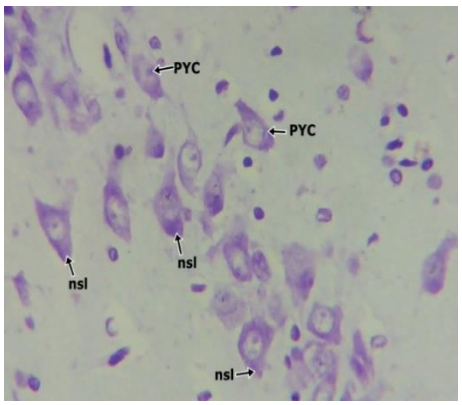

Plate 5: Photomicrograph of a section of hippocampus from group E shows evenly distributed and lightly stained Nissl granules.

Result of the present study revealed that the staining intensity was mild in the treated groups which may be due to the high content of the antioxidants, omega 3, zinc, magnesium, iron, copper which are very important for brain health. The omega 3 improves mental health and memory and supports a healthy brain development. Magnesium provides calming effects to the brain while zinc is very important in healthy functioning of the brain and other systems. Hence, zinc aids neurons in the hippocampus to communicate while omega 3 boasts cognitive function.[31] This study is also in line with reports where aqueous extract of *T. Occidentalis* possess potential effects against $HgCl_2$-induced oxidative stress and histological changes of rat hippocampus and cerebellum,[32] as well as aqueous *Averrhoa carambola* reducing GFAP-reactive astrocyte expression in diazepam-induced toxicity of the hippocampus in rats.[33] This restorative potential of the extract of *T. occidentalis* seeds could be attributed to the high polyphenols (antioxidants) content which may help neutralize excess free radicals, protect the cell against toxic effect as well as prevent further damage[16] from the SHB hence, providing an enabling environment for cells and tissues' survival.

Conclusion

Aqueous *T. occidentalis* seeds extract has the potential to restore Nissl bodies of hippocampal pyramidal cells and enhance short term memory caused by scopolamine hydrobromide in adult Wistar rats.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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